

Antimicrobial Resistance Patterns and Plasmid Profiles of *Staphylococcus Aureus* Isolated from Different Clinical Specimens in Saudi Arabia

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Abstract

The aims of this study were to investigate antibiotic resistance patterns and plasmid profile of antibiotic resistant *Staphylococcus strains* isolated from clinical specimens, and to find out a possible relationship between plasmids profile and antibiotic sensitivity patterns. *Staphylococcus* strains were isolated from different clinical specimens from different hospitals and primary health care centers. Antimicrobial susceptibility of *Staphylococcus* isolates was determined using disc diffusion method against 10 commonly used antimicrobial drugs. Plasmid DNA was extracted from lysed *Staphylococcus* cells using Plasmid Miniprep kit, and visualized using Agarose gel electrophoresis. The results showed that isolated strains of *Staphylococcus* were resistant to Penicillin (61%), Erythromycin (45%), Chloramphenicol (21%) and Co-trimoxazole (15%). Plasmid analysis of clinical isolates showed the presence of 0 to 3 plasmids with size range of 2 to 31 Kb, as compared to control *Staphylococcus aureus* ATCC 25923 which showed 4 plasmids (size range of 2 to 21 Kb). The results obtained in this study showed no direct correlation between the patterns of antibiotic resistance and plasmid profiles.

Keywords: Antibiotic resistance, Plasmids, *Staphylococcus aureus* Saudi Arabia

Introduction

Staphylococcus aureus (*S. aureus*) are part of the indigenous microflora of humans and animals, they are found on skin, nasal passages and mucous membrane (Kluytmans *et al.*, 1997). *S. aureus* is present in about 25-30% of humans especially healthy adults (Lowy 1998; Akpaka *et al.*, 2006; Heijer *et al.*, 2013) *S. aureus* is one of the major pathogen that causes skin, wound and burn infections, and other infections, septicemia and

endocarditis, antibiotic resistance is wide spread in these bacteria and may impact human health. It has been reported that hospital acquired (nosocomial) and community acquired staphylococcal infections may be resistant to as many as 20 antimicrobial agents, including antiseptics and disinfectants (Lyon and Skurray 1987). This resistance may result in part from acquisition of new genetic materials either through plasmids or via transposable genetic elements such as transposons or mutations within chromosomal genes (Cirz *et al.*, 2005; Harbottle *et al.*, 2006)

Plasmids are carried by bacteria as extra chromosomal, self-replicating genetic elements. They exist as double stranded, circular DNA molecule capable of self-replication. Plasmids do not usually carry genes essential for the growth of bacterial cells but the genes they carry may be expressed under stressed conditions (Thomas and Nielsen 2005).

The antibiotic resistant genes in *S. aureus* are usually found on plasmids (Sambrook *et al.*, 1989). Resistant strains of *S. aureus* to most antibiotics have risen in recent years, and only few antibiotics are still effective against staphylococci which include glycopeptides antibiotic such as vancomycin and teichoplanin (Chiquet *et al.*, 2007; Dar *et al.*, 2006; Tarazi *et al.*, 2015).

Studies carried out to determine the role of plasmids on antibiotic resistance has been useful in determining the characteristics of plasmids in bacteria (Jaran 2015; 2016). Plasmids can be transferred from one close bacterium to another horizontally, while for bacteria that are distant from one another plasmids can be transferred phylogenetic (Dale and Park 2004).

The aims of the present study were to investigate the antibiotic resistance patterns and plasmid profile of antibiotic resistant *S. aureus* strains, isolated from different clinical sources in Riyadh area Saudi Arabia, and to find a possible correlation between plasmids and antibiotic sensitivity patterns.

Material and methods

Bacterial Isolates

Forty *S. aureus* bacteria were isolated from different clinical sources (urine, blood, pus, peritoneal fluid, sputum, abscess and wounds) and transported to the microbiology laboratory at the Medical School, Al Imam Mohammed Bin Saud Islamic University, Riyadh, Saudi Arabia.

All isolates were identified using routine laboratory procedures. Gram stain, basic colonial morphology, cultural characteristics on MSA (i.e. reduced pH turned medium colour from red to yellow), standard biochemical tests, Catalase and coagulase tests. Isolates that were Gram-positive cocci (grape-like cluster), positive to catalase test, and slide coagulase test (Staphytec plus, Oxoid Diagnostic Reagents) were considered as *S. aureus*.

Antimicrobial Susceptibility

Antimicrobial susceptibility of *Staphylococcus* isolates was determined using disc diffusion method against 10 commonly used antimicrobial drugs in Saudi Arabia, according to the guidelines of CLSI (2015) on Mueller Hinton Agar plates (CLSI. 2015). Control strains of *S. aureus* (ATCC 25923) were used in the study. Single colonies were selected and Mueller Hinton broth was inoculated and incubated over night at 37° C, then the bacterial suspension adjusted to 0.5 McFarland standard. Bacterial lawns were spread on Muller Hinton Agar plates and antibiotic discs were placed on the surface of the agar and incubated at 37° C for 18hrs. The antimicrobial agents and their disc concentration were: Penicillin G, (P), (10 unites); Ciprofloxacin, (CIP), 5µg; Sulphamethoxazole-trimethoprim (Co-trimoxazole), (SXT), 25µg., Nalidixic acid, (NA), 30µg; Amikacin, (AMK), 30µg; Cephalothin, (KF), 30µg; Erythromycin, (E), 15µg; Clindamycin, (CD), 2µg; Oxacillin, (OX), 1µg; Chloramphenicol, (CHL), 30µg.

The size of the area of suppressed growth (zone of inhibition) was determined by the concentration of the antibiotics present in the area and, therefore, the diameter of the inhibition zone denotes, the relative susceptibility to a particular antibiotic. The interpretation of the results as sensitive or resistant was determined according to standard charts provided by the manufactures (OXOID Limited, Basingstoke, Hampshire, England).

Plasmid Isolation

Out of the 40 identified *S. aureus* isolates obtained, plasmid profiling was carried out on 15 randomly selected *S. aureus* isolates. The selected bacterial strain, (single colony) was grown overnight in Luria-Bertani (LB) broth at 37o C with aeration using an orbital shaker and plasmid DNA was extracted from lysed bacterial cells using Plasmid Miniprep kit from Promega Corporation (USA).

Agarose gel electrophoresis of plasmid DNA

Electrophoresis was carried out in a horizontal gel apparatus (Scie-Plas limited, Southam, Warwickshire, United Kingdom). Electrophoresis was conducted in agarose (0.8%) gel (Fisher Biotech, New Jersey, USA) and stained with ethidium bromide. The approximate molecular weight of plasmids (in mega Daltons) was determined by comparing with Lambda DNA Hind III digest (Promega-USA) as a standard marker.

Statistical Analysis

The correlation of plasmids to antibiotic resistance was calculated using Microsoft Excel (2013) programme.

Results and discussion

The *S. aureus* strains isolated from clinical samples showed that two isolates were resistant to six antibiotics, four isolates were resistant to five antibiotics, five isolates were resistant to four antibiotics and isolates resistant to three antibiotics accounted to 48%, isolates resistant to two antibiotics accounted to 21%, isolates resistant to one antibiotic accounted to 18% and 12% of total isolates were sensitive to all antibiotics tested.

Clinical isolates showed resistance mostly to Penicillin (61%), Erythromycin (45%), Chloramphenicol (21%) and Co-trimoxazole (15%) (Fig. 1).

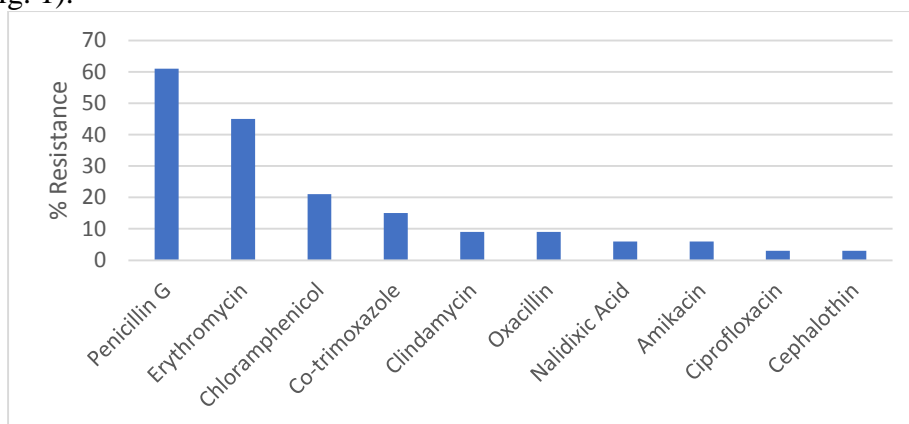


Fig. 1. Percentage resistance of antibiotics used against *Staphylococcus aureus* isolated from different clinical samples

The antibiotic resistance to all *Staphylococcus* strains isolated in this study are shown in Table 1.

Table 1. Antibiotic resistance to all *Staphylococcus* strains isolated in this study.

Antibiotics	Number of resistant isolates (%)
Penicillin G	24 (61)
Erythromycin	18 (45)
Chloramphenicol	8 (21)
Co-trimoxazole	6 (15)
Clindamycin	4 (9)
Oxacillin	4 (9)
Nalidixic Acid	3 (6)
Amikacin	3 (6)
Ciprofloxacin	2 (3)
Cephalothin	2 (3)

Plasmid analysis of clinical isolates showed the presence of 1 to 3 plasmids with size range of 2 to 31 Kb in 7 of the isolates. Seven of the randomly selected isolates showed no plasmids, as compared to control *S.*

aureus ATCC 25923 which showed 4 plasmids with size range of 2 to 21 Kb (Table 2).

Table 2. Plasmid characterization isolated from *Staphylococcus* strains, showing numbers and sizes of plasmids and pattern of resistant antibiotics.

Isolate No.	Clinical Source	Number of Plasmids Isolated	Size of Plasmid (Kb)	Resistant Antibiotics
1	Urine	3	31.0, 21.0, 2.0	SXT, NA, E
2	Swab	2	21.0, 4.0	P, SXT, E, CHL
3	Peritoneal fluid	1	21.0	CD, E, OX, P
4	Abscess	3	31.0, 21.0, 2.0	P, CHL, E
5	Pus	3	21.2, 4.2, 2.2	P, CHL, E
6	Blood	1	4.0	-
7	Wound	2	21.0, 4.2	P, SXT, E, CHL
8	Pus	No plasmids detected	-	E, P, SXT
9	Abscess	No plasmids detected	-	CHL, P
10	Sputum	No plasmids detected	-	P
11	Swab	No plasmids detected	-	-
12	Pus	No plasmids detected	-	P, CHL
13	Pus	No plasmids detected	-	AMK, AZT, CIP, E
14	Blood	No plasmids detected	-	KF, OX, CHL, P, E
15	Urine	No plasmids detected	-	P, NA, E
16	ATCC 25923	4	21.0, 4.5, 3.5, 2.0	-

Antibiotic resistance by pathogenic bacteria especially those that cause nosocomial infections including *S. aureus* present a big challenge for doctors.

This study has revealed that *S. aureus* isolated from different clinical specimens (urine, blood, pus, peritoneal fluid, sputum, abscess and wounds) showed varying patterns of resistance to the antibiotics used with penicillin G and Erythromycin being the most resistant (61% and 45% respectively). Multidrug-resistant (MDR) *S. aureus* that showed resistance to three or more different classes of antibiotics have been identified in this study, the isolated strains displayed an MDR phenotype against several antibiotics; Penicillin G (61%), Erythromycin (45%), Chloramphenicol (21%) and Co-trimoxazole (15%). These findings agree with several reports on *S. aureus* resistance patterns (Tarazi *et al.*, 2015; Adegoke and Okoh 2011; Bashir *et al.*, 2007;

Yasmeen *et al.*, 2010) which showed that resistance to Erythromycin was between 51% to 95%, other reports from Jordan showed that Amikacin, Chloramphenicol and Ciprofloxacin were sensitive to the isolated *S. aureus*, but resistance to Penicillin was 63% (Tarazi *et al.*, 2015), and Clindamycin 1.5% (Shehabi *et al.*, 2013). A study in 2010 from Italy reported similar results to our findings, high resistance rates to penicillin, gentamicin, erythromycin, clindamycin and ciprofloxacin and 19.1% of the isolated *S. aureus* from hospitals were susceptible to all antibiotics tested (Rea *et al.*, 2010).

Plasmids play an important role in mediating bacterial resistance to antimicrobial agents, they act as vectors of resistance genes that lead to rapid spread of antibiotic resistance as well as transferring resistance in bacterial populations including *S. aureus* (Lacey 1975). Our study has demonstrated different plasmid profiles in the isolated strains of *S. aureus* which did not correlate to the pattern of resistance observed. Resistance was observed in isolates with various molecular size plasmids as well as in those that had no plasmids (Table 2), also no particular molecular size plasmid could be associated with any particular antimicrobial resistance patterns. The number and size of plasmids isolated from the *S. aureus* strains used in this study did not show any correlation to antibiotic resistance ($r = 0.3858$). This could be explained by the fact that plasmids may be randomly distributed in the isolated *S. aureus*, or the resistance genes could be located on either the plasmids or chromosomes or the fact that the genes conferring resistance may be found in more than one plasmid with various size which is in agreement with a study conducted in Nigeria where they found that no relation were found between resistance patterns and plasmid profiles in *S. aureus* isolates from human samples (Chibuike *et al.*, 2014).

Conclusion

The isolated *Staphylococcus* strains showed resistance to 1 to 6 different antibiotics, Penicillin G being the most resistant antibiotic found in this study (61%). The isolated strains found to contain various numbers of plasmids (0 to 3) with different sizes (2 to 31 Kb). No direct correlation was found between the number of plasmids isolated from *Staphylococcus* strains and the number of antibiotics found to be resistant to these strains. The collection of more bacterial isolates from various sources and the addition of other tools for genetic analysis such as DNA sequencing of isolated plasmids should provide more information on the dynamics of the introduction and spread of antibiotic resistant bacteria in nature.

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